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## Plasma linoleic acid levels and cardiovascular risk factors

*results from the Norwegian ACE 1950 Study*

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# **Plasma linoleic acid levels and cardiovascular risk factors: Results from the Norwegian ACE 1950 Study**

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**Running title:**

Linoleic acid and cardiovascular risk factors

**Abbreviations:**

ACE	Akershus Cardiac Examination
BMI	Body mass index
cIMT	Carotid intima-media thickness
CI	Confidence interval
CRP	C-reactive protein
CV	Cardiovascular
DM	Diabetes mellitus
eGFR	Estimated glomerular filtration rate
HbA1c	Glycated hemoglobin
HDL	High-density lipoprotein
LA	Linoleic acid
LDL	Low-density lipoprotein
PUFA	Polyunsaturated fatty acid
Std. $\beta$ -coeff.	Standardized regression coefficient
Unstd. $\beta$ -coeff.	Unstandardized regression coefficient
wt%	Weight percentage

## **Abstract**

**Background:** A high intake of linoleic acid (LA), the major dietary polyunsaturated fatty acid (PUFA), has previously been associated with reduced cardiovascular (CV) morbidity and mortality in observational studies. However, recent secondary analyses from clinical trials of LA-rich diet suggest harmful effects of LA on CV health.

**Methods:** A total of 3,706 participants, all born in 1950, were included in this cross-sectional study. We investigated associations between plasma phospholipid levels of LA and CV risk factors in a Norwegian general population, characterized by a relative low LA and high marine n-3 PUFA intake. The main statistical approach was multivariable linear regression.

**Results:** Plasma phospholipid LA levels ranged from 11.4 to 32.0 wt%, with a median level of 20.8 wt% (interquartile range 16.8-24.8 wt%). High plasma LA levels were associated with lower serum low-density lipoprotein cholesterol levels (Standardized regression coefficient [Std.  $\beta$ -coeff.] -0.04,  $p=0.02$ ), serum triglycerides (Std.  $\beta$ -coeff. -0.10,  $p<0.001$ ), fasting plasma glucose (Std.  $\beta$ -coeff. -0.10,  $p<0.001$ ), body mass index (Std.  $\beta$ -coeff. -0.13,  $p<0.001$ ), systolic and diastolic blood pressure (Std.  $\beta$ -coeff. -0.04,  $p=0.03$  and Std.  $\beta$ -coeff. -0.02,  $p=0.02$ , respectively) and estimated glomerular filtration rate (Std.  $\beta$ -coeff. -0.09,  $p<0.001$ ). We found no association between plasma LA levels and high-density lipoprotein cholesterol levels, glycated hemoglobin, carotid intima-media thickness or C-reactive protein.

**Conclusion:** High plasma LA levels were favorably associated with several CV risk factors in this study of a Norwegian general population.

## Introduction

Linoleic acid (LA) is an essential fatty acid, mainly derived from vegetable oils, nuts, eggs and meats (1). It is the major n-6 polyunsaturated fatty acid (PUFA) and the most abundant PUFA in the Western diet (1). LA intake has previously been associated with a lower risk of cardiovascular (CV) morbidity and mortality in large epidemiological studies (2-4). However, recent published secondary analysis from the Sydney Diet Heart Study and Minnesota Coronary Experiment have raised concerns, as they report a direct association between diets rich in LA and an increase in overall and coronary heart disease mortality (5, 6). One proposed mechanism for this observation is an LA-induced increase in oxidized low-density lipoprotein (LDL) cholesterol particles, which are highly atherogenic (5, 6). LA has also been suggested to promote inflammation by serving as a precursor for arachidonic acid, an n-6 PUFA that is oxidized to proinflammatory cytokines during eicosanoid metabolism (7). In addition, LA reduces the conversion of plant derived n-3 PUFA alpha-linolenic acid to the anti-inflammatory n-3 PUFAs eicosapentaenoic and docosahexaenoic acid (7), also found in fatty fish and other seafoods.

The effects of LA intake on CV health have therefore been a matter of discussion and have resulted in inconsistent recommendations on LA consumption across regions. While the American Heart Association recommends consumption of at least 5% to 10% energy from LA intake, the European Food Safety Authority, supported by the French national guidelines, recommend 4% (8-10). A recent systemic review, evaluating LA intake in various European nations, showed that the European Food Safety Authority recommendations were not met in half of the countries included in the study (11). Compared to a modern Western diet, the traditional Nordic diet is characterized by a relatively high marine n-3 PUFA and low LA consumption (12).

Thus, there is a possibility that the LA intake might be lower than optimal also in Norway. The average energy intake from total PUFAs (the sum of n-3 PUFAs and n-6 PUFAs) was approximately 6% in Norway in 2012 (13), hence the energy intake from LA alone was probably lower than American Heart Association's recommendations. The assessment of how a marker of LA consumption is associated with the CV risk profile in a Norwegian general population might add new knowledge on the relationship between LA intake and CV health. The objective of this study was to investigate associations between plasma LA levels, as an estimate of LA intake (14, 15), and multiple CV risk factors.

## **Materials and methods**

### **Study population**

The Akershus Cardiac Examination (ACE) 1950 Study is a prospective population-based cohort study, evaluating the cardio- and cerebrovascular health of men and women born in 1950 and living in Akershus County, Norway. The study design and baseline examinations of the ACE 1950 Study have previously been described in detail (16). In brief, the Norwegian population register was used to identify all eligible individuals in Akershus County, who were invited for study participation by letters and subsequent phone calls. Among 5,827 invited individuals, 3,706 agreed to participate (64%) (Figure 1). The remaining 2,121 did not respond to the study invitation letter or declined participation. Enrollment was performed at Akershus University Hospital and Bærum Hospital from September 2012 through May 2015. In this article, we present data from a cross-sectional analysis of the baseline examination.

### **Data collection and procedures**

All participants completed questionnaires on medical history, diet, education, physical activity and smoking. Higher education was defined as >12 years of formal education.

Smoking habits were classified as current smoker or previous/non-smoker. High physical activity was defined as >2 sessions of exercise per week. Alcohol consumption was defined as intake of alcohol  $\geq 2$  times per week. Study participants underwent a standardized physical examination. Hypertension was defined as mean systolic blood pressure  $\geq 140$  mmHg or mean diastolic blood pressure  $\geq 90$  mmHg from three measurements in the sitting position, or current use of anti-hypertensive medication. The World Health Organizations definition was used to define obesity (body mass index [BMI,  $\text{kg/m}^2$ ]  $\geq 30$ ) (17).

Blood samples were obtained after overnight fasting and stored at  $-80^\circ\text{C}$  until analysis.

Hypercholesterolemia was defined as total serum cholesterol  $\geq 6.2$  mmol/L or LDL cholesterol  $\geq 4.1$  mmol/L (18), or current use of lipid-lowering agents. Diabetes mellitus (DM) was defined as both glycated hemoglobin (HbA1c)  $\geq 6.5\%$  and fasting plasma glucose  $\geq 7.0$  mmol/L, self-reported DM or current use of glucose-lowering medications. Chronic kidney disease stages 3-5 were defined as estimated glomerular filtration rate (eGFR)  $<60$  ml/min/1.73m<sup>2</sup> using The Chronic Kidney Disease Epidemiology Collaboration equation (19). Standard assay was used for the measurement of C-reactive protein (CRP).

Carotid intima-media thickness (cIMT) was assessed by ultrasound examination of the right and left carotid arteries, as previously described (20). Mean cIMT was obtained from the average of right and left cIMT measurements.

### **Plasma phospholipid fatty acid measurement**

Aliquots from stored frozen blood samples were sent to The Lipid Research Center, Aalborg University Hospital for plasma fatty acid analysis by gas chromatography. In brief, Folch method was used to extract total lipids from serum (21), and the Burge method was used to isolate the phospholipid fraction from other lipids (22). The fatty acids were analyzed by a Varian 3900 gas chromatograph (Varian, Middleburg, The Netherlands) with 60 m x 0.25 mm

capillary columns. Individual fatty acids were identified from their retention time during gas chromatography, and quantified as weight percentage (wt%) of total plasma phospholipid fatty acids.

Data on plasma LA levels were missing in 23 participants, either due to insufficient plasma volume (n=17) or inadequate plasma fatty acid analysis (n=6) (Figure 1).

### **Ethical considerations**

All participants gave written informed consent before final enrollment. The study was approved by the Norwegian Regional Ethics Committee (September 7<sup>th</sup> 2011. Ref. number 2011/1475) and performed in accordance with the Declaration of Helsinki. Registered at clinicaltrials.gov, registration number NCT01555411.

### **Statistical analysis**

Quartiles of plasma LA were used for presentation of demographic and clinical data. Results are presented as mean values (standard deviations) for normally distributed data, median values (inter quartile ranges) for skewed data (serum triglycerides, fasting plasma glucose, HbA1c and CRP) and percentages for categorical data. Between-groups differences were assessed by Chi square test for dichotomous variables, Kruskal-Wallis test for skewed variables and ANOVA for normally distributed continuous variables. Bivariate associations between plasma LA levels and plasma eicosapentaenoic and docosahexaenoic acid levels, higher educational level, alcohol consumption, gender, smoking and physical activity are presented with Spearman's correlation ( $r_s$ ). We investigated multivariable adjusted associations between plasma LA levels and CV risk factors, after testing for linearity with standard methods and restricted cubic spline modelling, the graphical presentation for which is presented in Figure 2. For restricted cubic spline modelling, the continuous variable plasma



LA levels was subdivided by use of three knots, placed at 10<sup>th</sup>, 50<sup>th</sup> and 90<sup>th</sup> percentile of the exposure distribution. Although assumptions for linearity between plasma LA levels and serum LDL cholesterol and HbA1c were not fully met, multivariable linear regression was performed for these variables. Adjustments were made with for each CV risk factor with covariates chosen from a set of predefined candidate variables by least absolute shrinkage and selection operator (LASSO) regression (Supplementary table 1). First highly inflated Std.  $\beta$ -coeff. were penalized towards zero. A tuning parameter lambda, which was chosen for each CV risk factor after cross validation, controlled the strength of penalty. Second, during variable selection, candidate variables with regression coefficients  $> 0$  after the shrinkage process, were included in the final model. All models were constructed using simultaneous entry of variables. Skewed variables were truncated to obtain normal distribution, before they were entered into the regression models. Because of extreme skewness, CRP was logarithmically transformed before it was entered as a variable in the regression analyses. Thus, for CRP, the presented unstandardized regression coefficients (Unstd.  $\beta$ -coeff.) and corresponding 95% confidence intervals (CI) represent the anti-logarithm of obtained results. The table displays Unstd.  $\beta$ -coeff. with corresponding 95% CI, standardized regression coefficients (Std.  $\beta$ -coeff.), p-values and explained variance ( $R^2$ ) for the univariable and the fully adjusted final models. All statistical calculations were performed using SPSS® version 25.0 (IBM, NY, US), RStudio® version 1.1.419 and R version 3.4.4 (RStudio Inc., Boston, MA, US and R Foundation for Statistical Computing, Vienna, Austria).

## **Results**

### **Demographics**

Demographics and clinical characteristics of study participants are presented in Table 1.

Plasma LA levels ranged from 11.4 to 32.0 wt%, with a median level of 20.8 wt%

(interquartile range 16.8 to 24.8 wt%). Participants with high plasma LA levels had lower plasma levels of eicosapentaenoic and docosahexaenoic acid ( $r_s = -0.44$  and  $r_s = -0.37$ ,  $p < 0.001$  for both). Higher education ( $r_s = 0.07$ ,  $p < 0.001$ ) and less alcohol consumption ( $r_s = -0.07$ ,  $p < 0.001$ ) were seen in participants with high plasma LA levels, while no differences were observed for gender ( $r_s = -0.01$ ,  $p = 0.62$ ), smoking ( $r_s = -0.03$ ,  $p = 0.09$ ) or physical activity ( $r_s = 0.03$ ,  $p = 0.13$ ). Furthermore, a lower prevalence of hypertension, hypercholesterolemia, cerebrovascular and coronary artery disease, DM and obesity were also seen in participants with high compared to low levels of plasma LA. Moreover, the use of antihypertensive-, lipid-lowering and glucose-lowering medications were lower in participants with higher plasma LA levels.

### **Plasma LA levels and CV risk factors**

Cubic spline modeling of associations between plasma LA levels and CV risk factors are presented in Figure 2. Except for serum LDL-cholesterol levels and HbA1c, the associations between plasma LA levels and CV risk factors were linear. Unadjusted and multivariable adjusted associations between plasma LA levels and CV risk factors are presented in Table 2. High plasma LA levels were associated with lower serum LDL cholesterol and triglyceride levels, while no association was found with serum high-density lipoprotein (HDL) cholesterol levels.

High plasma LA levels were associated with lower fasting plasma glucose. When stratifying participants according to DM, plasma LA levels showed a statistically significant association fasting plasma glucose in participants without DM ( $n = 3296$ , Unstd.  $\beta$ -coeff.  $-0.03$  [95% CI  $-0.03, -0.02$ ], Std.  $\beta$ -coeff.  $-0.13$ ,  $p < 0.001$ ), while a trend was seen in participants with DM ( $n = 309$ , Unstd.  $\beta$ -coeff.  $-0.05$  [95% CI  $-0.10, 0.02$ ], Std.  $\beta$ -coeff.  $-0.11$ ,  $p = 0.15$ ). We found no associations between plasma LA levels and HbA1c.

Plasma LA levels were inversely associated with BMI and systolic and diastolic blood pressure (Table 2).

High plasma LA levels were associated with lower eGFR. Participants were stratified according to eGFR levels above or below 60 ml/min/1.73m<sup>2</sup>. Plasma LA levels were inversely associated with eGFR >60 ml/min/1.73m<sup>2</sup> (n=3452, Unstd.  $\beta$ -coeff. -0.28 [95% CI -0.40, -0.15], Std.  $\beta$ -coeff. -0.08, p<0.001). In contrast, no association was found with eGFR <60 ml/min/1.73m<sup>2</sup> (n=141, Unstd.  $\beta$ -coeff. -0.01 [95% CI -0.47, 0.46], Std.  $\beta$ -coeff. -0.003, p=0.98).

We found no statistical association between plasma LA levels and cIMT or CRP (Table 2).

When stratifying participants according to educational level and alcohol consumption, associations between plasma LA levels and CV risk factors did not change (data not shown).

## **Discussion**

The main finding of the present study was that high levels of plasma LA were associated with lower fasting plasma glucose and BMI. High plasma LA levels were associated with lower serum triglyceride levels and systolic blood pressure. We found no association between plasma LA levels and CRP.

### **Plasma LA levels and CV risk factors**

#### **Plasma LA levels and fasting plasma glucose, HbA1c and BMI**

In the present study, we found a lower prevalence of DM among participants in the upper quartile of plasma LA levels. High levels of plasma LA were associated with lower fasting plasma glucose in individuals without DM, while no statistically significant association was

found in participants with DM, in whom most received glucose-lowering medication that could possibly confound results. In a pooled analysis of 20 prospective studies, including >39,000 participants from 10 different countries, high plasma levels of LA were associated with a lower risk of incident type 2 DM (23). These reports, along with our findings, may suggest that LA consumption might contribute in prevention of DM. Surprisingly, plasma LA levels were not associated with HbA1c. Similar results have been reported in a cross-sectional study from the Dutch general population, aged 50-74 (24). Concordant with our findings, they found that high plasma LA levels were associated with lower fasting plasma glucose, while no associations were found with HbA1c (24). The underlying mechanism by which LA influences the glucose-insulin homeostasis is poorly understood. Improved insulin sensitivity mediated by LA has been suggested (25). Another possible mechanism could be increased glucose consumption related to the upregulation of carnitine shuttle system required for mitochondrial  $\beta$ -oxidation of long-chain fatty acids, such as LA (26). Increased mitochondrial  $\beta$ -oxidation after a high intake of long-chain fatty acids has also been suggested to induce weight loss (27), which could partly explain the lower prevalence of obesity and lower BMI associated with high plasma LA levels in the present study. On the other hand, weight loss achieved by increased LA intake in clinical trials has been at the expense of energy from carbohydrates (27), and can therefore not be attributed to LA intake alone.

In addition to LA consumption, plasma LA levels are influenced by delta-6 desaturase activity regulating the endogenous LA metabolism (28). In a population-based cohort study, participants with metabolic syndrome and insulin resistance had higher delta-6 desaturase activity and lower serum LA levels compared to control (29). A higher delta-6 desaturase activity has also been associated with insulin resistance (30). Hence, we cannot rule out

reverse causality bias that could possibly underlie inverse associations between plasma LA levels and fasting plasma glucose levels.

### **Plasma LA levels and CRP**

A concern for harm from a high LA intake has been linked to the potential conversion of LA to arachidonic acid, a precursor for inflammatory cytokines. However, this endogenous conversion is tightly regulated and has been reported to be less than 0.3% in a tracer study with stable isotopes (31). In a systematic review of human trials, a 6-fold increase in LA consumption did not affect plasma or tissue concentration of arachidonic acid (32).

Furthermore, a high LA intake in clinical trials did not increase inflammatory biomarkers such as CRP, interleukin-6, tumor necrosis factor- $\alpha$  or plasma fibrinogen (33). In the present study, plasma LA levels were not associated with CRP. Thus, our finding does not support the proposed proinflammatory nature of LA. It should however be mentioned that we did not measure high-sensitive CRP, which is a more reliable marker of low-grade inflammation, hence our finding should be interpreted with caution with regards to CRP levels. Furthermore, the median CRP level was 1.5 mg/L in our cohort, a level which has been associated with an average risk of CV disease among Caucasians in other community-based studies (34).

Advice to reduce LA intake has also been based on the competition with n-3 PUFAs, as LA theoretically can inhibit the conversion of alpha-linolenic acid to eicosapentaenoic acid and docosahexaenoic acid (7). However, in humans this conversion is low (<5%) (35), and likely of minor importance in a Norwegian general population with a high background consumption of marine n-3 PUFAs and a low alpha-linolenic acid intake (12).

### **Plasma LA levels and serum lipids**

The relationship between LA and blood lipids is not clearly understood. In a meta-analysis of 60 clinical studies, the substitution of LA for carbohydrates reduced serum LDL cholesterol and triglyceride levels (36). Similarly, substituting saturated fatty acids with LA, in a clinical trial in men with dyslipidemia, resulted in a decreased production of LDL particles (37).

These reports indicate possible lipid-lowering properties of LA, but should be interpreted with caution as they were obtained at the expense of carbohydrates and saturated fatty acids. One suggested mechanism by which LA influences blood lipids is increased clearance of triglyceride rich particles, the upstream of LDL cholesterol production (37). Furthermore, a high intake of LA has shown to increase lipoprotein lipase activity in experimental studies (38), an enzyme involved in hydrolysis of triglycerides. In the present study, high plasma LA levels were associated with lower serum LDL cholesterol and triglyceride levels.

Furthermore, participants in the upper compared with the lower quartile of plasma LA levels had a lower prevalence of hypercholesterolemia and used lipid-lowering drugs less often.

However, the inverse associations between plasma LA levels and serum LDL cholesterol in the present study must be interpreted with caution for several reasons. First, assumptions for a linear association between plasma LA and serum LDL cholesterol levels were not met.

Second, participants in the upper compared to lower quartile of plasma LA levels had higher serum LDL cholesterol levels. Finally, in our cohort, plasma LA and serum LDL cholesterol levels seemed to have an inverted U-shaped relationship (Figure 2), which does not appear biological plausible. Thus, we speculate that this association might have been confounded by factors we have not been able to identify and adjust for in our analyses.

Plasma LA levels were not associated with serum HDL cholesterol levels, consistent with a recent meta-analysis of clinical trials, reporting no effect of LA intake on HDL cholesterol (39).

### **Plasma LA levels and cIMT, blood pressure and kidney function**

cIMT is a validated surrogate marker of future CV events (40). The association between serum LA and cIMT was evaluated in a Spanish population, with a high background consumption of vegetable oils as a part of the Mediterranean diet (41). In that cross-sectional study, serum LA levels were not associated with cIMT. Similarly, in our cohort, with a low background intake of LA, we did not find an association with cIMT. Furthermore, we found only a weak association between plasma LA levels and blood pressure, and no association with serum HDL cholesterol levels, which are considered strong predictors of cIMT progression (42, 43). Finally, a recent meta-analysis reported no effect on blood pressure from LA intake (39).

Data on LA intake and kidney function are scarce. A prospective cohort study found less decline in renal function at 3-year follow-up in participants with higher plasma LA levels at baseline (44). In the present study, plasma LA levels were not associated with eGFR  $<60$  ml/min/1.73m<sup>2</sup>, suggesting no benefits for renal function in participants with chronic kidney disease stages 3-5.

### **Plasma LA levels and general health and lifestyle**

Participants in the upper quartile of plasma LA levels were generally healthier than the remaining cohort, with a lower prevalence of hypertension, hypercholesterolemia, DM, obesity, cerebrovascular and coronary artery disease. This was also reflected by a lower use of antihypertensive-, lipid-lowering and glucose-lowering medications among these participants. Plasma LA levels were not associated with factors such as smoking habits or physical activity, and associations with CV risk factors remained unchanged when participants were stratified according to educational level and alcohol consumption. Thus, the findings signal that high plasma LA levels may favorably influence CV risk factors. High plasma LA levels

were associated with overall healthier lifestyle producing a confounder effect, for which we attempted to adjust for in the statistical analysis. However, residual confounding from variables not included in the regression models might still have influenced results, leading to a possible overestimation of associations between plasma LA levels and CV risk factors. Low plasma LA levels have previously been associated with higher BMI, fasting plasma glucose, triglycerides and alcohol consumption (45). Similar findings were reported in statin users compared to non-users (46). A possible explanation could be altered plasma fatty acid composition in individuals with adverse metabolic risk profile, in which case plasma LA level might serve as a marker of cardiometabolic health. Thus, in the present study, we cannot rule out the possibility that our findings have been influenced by reverse causality bias.

### **Strengths and limitations**

In this study we measured plasma LA levels as an estimate of LA intake, which is a more reliable marker than self-reported intake from dietary interviews, which are often influenced by recall bias. To our knowledge, this is the largest cohorts in a Norwegian general population where plasma LA levels have been measured by gas chromatography. The study population was well-described and had little missing data. In addition, all participants were born in 1950, thus removing age as a confounding factor.

There are also several limitations. The cross-sectional study design does not allow for any conclusion on causality. Dietary data on LA consumption was not available for this study. Plasma phospholipid PUFA levels do not represent long-term fatty acid intake as accurately as erythrocyte membrane or adipose tissue PUFA levels. However, for LA level estimates, plasma is an equally representative compartment as erythrocyte membrane or adipose tissue (14). Despite careful adjustments for covariates, we cannot rule out residual confounding influencing associations between plasma LA levels and CV risk factors. Furthermore, we



acknowledge that our results are characterized by weak regression coefficients and that statistically significant associations between plasma LA levels and CV risk factors might have been driven by the large sample size. However, for the purpose of proposing hypothesis regarding the impact of LA consumption on CV health, we consider our results to be applicable to a Nordic general population. Our results might not apply to other regions, especially those with a high LA consumption. Finally, as all the participants in the ACE 1950 Study were born in 1950, our results might not apply to other age groups.

## **Conclusion**

We found favorable associations between high plasma LA levels and various CV risk factors in this cross-sectional study of Norwegian individuals born in 1950.

Well-designed clinical trials are needed to study the effect of an increased LA intake on CV risk factors.

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Finally, we would like to thank the study participants.

## **Disclosures**

The authors declare no disclosures or conflict of interest.

The results presented in this paper have not been published previously and is not under consideration for publication anywhere else.

## **Author Contributions**

A.C, I.A.E and M.S designed the present study.

H.R, T.O, M.N.L, T.B and A.T designed and organized the ACE 1950 Study including baseline examinations and data collection.

T.V, H.I.-H, E.B.O and O.M.R performed carotid ultrasound and baseline examinations.

E.B.S was responsible for the fatty acid analyses.

A.C, I.A.E and S.N analysed the data.

A.C, I.A.E and M.S edited the manuscript, E.B.S, T.O, H.R, T.V, H.I.-H, S.N, E.B.O, O.M.R, M.N.L, T.B and A.T co-edited the manuscript.

All the authors approved the final version of the manuscript.

A.C submitted the manuscript.

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## **Figure legends**

### **Figure 1**

Flowchart of the inclusion of study participants.

### **Figure 2**

Univariate associations between plasma linoleic acid levels (x-axis) and CV risk factors (y-axis), modeled as restricted cubic spline. We used three knots, placed at 10<sup>th</sup>, 50<sup>th</sup> and 90<sup>th</sup> percentile of plasma linoleic acid levels, measured in weight percentage of total plasma phospholipid fatty acids.

**Table 1. Characteristics of study participants according to quartiles of plasma linoleic acid levels.**

	All patients	Quartile 1	Quartile 2	Quartile 3	Quartile 4	p
<b>Linoleic acid level, wt%</b>	<b>11.4-32.0</b>	<b>≤18.74</b>	<b>18.75–20.82</b>	<b>20.83-22.77</b>	<b>≥22.78</b>	
Number of participants	3683	922	919	923	919	
Age, <i>months</i>	767 (7.8)	768 (7.9)	767 (7.7)	767 (7.9)	766 (7.4)	<0.001
Gender (Male), %	51.3	54.0	49.2	49.3	52.8	0.08
Daily fruit intake, %	51.2	51.2	52.3	50.6	50.7	0.89
Daily vegetable intake, %	58.9	58.6	58.5	60.8	57.9	0.61
Daily whole wheat bread intake, %	57.3	58.3	55.9	57.7	57.4	0.76
EPA, <i>wt%</i>	2.6 (1.4)	3.4 (1.7)	2.8 (1.2)	2.3 (1.1)	1.9 (0.9)	<0.001
DHA, <i>wt%</i>	5.5 (1.4)	6.2 (1.6)	5.7 (1.4)	5.3 (1.2)	4.8 (1.2)	<0.001
ALA, <i>wt%</i>	0.26 (0.1)	0.22 (0.1)	0.25 (0.1)	0.27 (0.1)	0.29 (0.1)	<0.001
AA, <i>wt%</i>	9.2 (2.1)	10.3 (2.4)	9.6 (1.9)	8.9 (1.6)	8.1 (1.6)	<0.001
Current smoker, %	14.5	16.7	13.2	15.0	13.0	0.10
Alcohol consumption, (≥ 2 times weekly), %	47.4	51.8	49.1	46.2	42.3	<0.001
Physical activity (≥ 2 times weekly), %	61.7	58.9	62.6	63.2	62.3	0.22
Higher education, %	46.5	42.8	43.5	48.1	51.6	<0.001
Systolic blood pressure, <i>mmHg</i>	138 (19)	139 (19)	139 (19)	137 (18)	137 (19)	0.01
Diastolic blood pressure, <i>mmHg</i>	77 (10)	78 (10)	77 (10)	77 (10)	76 (10)	0.11
Total cholesterol, <i>mmol/L</i>	5.4 (1.1)	5.2 (1.2)	5.5 (1.1)	5.6 (1.1)	5.5 (1.0)	<0.001
HDL cholesterol, <i>mmol/L</i>	1.5 (0.5)	1.5 (0.5)	1.5 (0.5)	1.6 (0.5)	1.6 (0.5)	<0.001
LDL cholesterol, <i>mmol/L</i>	3.3 (1.0)	3.0 (1.0)	3.4 (1.0)	3.4 (0.9)	3.4 (0.9)	<0.001
Triglycerides, <i>mmol/L</i>	1.2 (0.4-2.0)	1.3 (0.3-2.8)	1.2 (0.4-2.0)	1.2 (0.5-1.9)	1.0 (0.3-1.7)	<0.001
Fasting plasma glucose, <i>mmol/L</i>	5.3 (4.5-6.2)	5.5 (4.5-6.5)	5.3 (4.4-6.2)	5.3 (4.5-6.1)	5.2 (4.4-6.0)	<0.001
HbA1c, %	5.7 (5.3-6.1)	5.7 (5.2-6.2)	5.7 (5.3-6.1)	5.7 (5.3-6.1)	5.7 (5.3-6.1)	<0.001

Body mass index (BMI), <i>kg/m<sup>2</sup></i>	27.1 (4.4)	28.1 (4.4)	27.3 (4.3)	27.1 (4.5)	26.0 (4.3)	<0.001
eGFR, <i>ml/min x 1.73m<sup>2</sup></i>	83 (12.0)	84 (12.3)	83 (11.8)	84 (12.0)	82 (11.5)	0.008
cIMT, <i>mm</i>	0.73 (0.1)	0.74 (0.1)	0.73 (0.1)	0.73 (0.1)	0.72 (0.1)	0.01
CRP, <i>mg/L</i>	1.5 (1.5-1.5)	1.5 (1.5-1.5)	1.5 (1.5-1.5)	1.5 (1.5-1.5)	1.5 (1.5-1.5)	0.07
Hypertension, %	62.0	72.6	62.5	59.5	53.3	<0.001
Hypercholesterolemia, %	52.6	71.0	58.2	47.1	33.9	<0.001
Cerebrovascular disease, %	3.7	5.1	4.1	4.1	1.6	<0.001
Coronary artery disease, %	7.0	13.7	6.7	4.9	2.8	<0.001
Diabetes mellitus, %	8.5	12.9	9.4	6.3	5.4	<0.001
Obesity (BMI ≥ 30), %	22.6	28.5	24.4	22.2	15.1	<0.001
CKD stages 3-5 (eGFR <60 <i>ml/min x 1.73m<sup>2</sup></i> ), %	3.9	3.9	3.6	3.4	4.6	0.58
Medication, %						
Diuretics	3.1	4.3	3.2	2.4	2.4	0.05
Beta blockers	13.4	20.8	13.2	11.4	8.1	<0.001
Calcium channel blockers	8.1	12.1	7.7	8.8	3.9	<0.001
ACEi or ARB	26.9	36.2	26.3	25.6	19.6	<0.001
Lipid-lowering drugs	26.1	49.9	27.9	17.2	9.6	<0.001
Glucose-lowering drugs	5.2	8.2	4.8	4.5	3.2	<0.001

Results are presented as percentage for categorical data and mean value (standard deviation) for continuous data. Differences between groups were evaluated using Chi square for dichotomous data, the Kruskal-Wallis test for triglycerides, fasting plasma glucose, HbA1c and CRP, and ANOVA for other continuous data.

Abbreviations: EPA: Eicosapentaenoic acid. DHA: Docosahexaenoic acid. ALA: Alpha-linoleic acid. AA: Arachidonic acid. HDL: High density lipoprotein. LDL: Low density lipoproteins HbA1c: Hemoglobin A1c. BMI: Body mass index. eGFR: Estimated glomerular filtration rate (CKD-EPI formula). cIMT: Carotid intima-media thickness. CRP: C-reactive protein. CKD: Chronic kidney disease. ACEi: Angiotensin converting enzyme inhibitor. ARB: Angiotensin receptor blocker.

**Table 2. Associations between plasma linoleic acid levels and cardiovascular risk factors.**

Univariable linear regression analysis					
Cardiovascular risk factors	n	Unstd. $\beta$ -coeff. (95% CI)	Std. $\beta$ -coeff.	p	R <sup>2</sup>
HDL cholesterol, <i>mmol/L</i>	3680	0.01 (0.01, 0.02)	0.08	<0.001	0.01
LDL cholesterol, <i>mmol/L</i>	3657	0.05 (0.04, 0.06)	0.14	<0.001	0.02
Triglycerides, <i>mmol/L</i>	3680	-0.04 (-0.05, -0.03)	-0.18	<0.001	0.03
Fasting glucose, <i>mmol/L</i>	3675	-0.06 (-0.07, -0.05)	-0.18	<0.001	0.03
HbA1c, %	3669	-0.02 (-0.02, -0.01)	-0.08	<0.001	0.01
BMI, <i>kg/m<sup>2</sup></i>	3683	-0.27 (-0.31, -0.23)	-0.18	<0.001	0.03
SBP, <i>mmHg</i>	3679	-0.40 (-0.61, -0.20)	-0.06	<0.001	0.004
DBP, <i>mmHg</i>	3679	-0.12 (-0.23, -0.01)	-0.04	0.03	0.001
eGFR, <i>ml/min x 1.73m<sup>2</sup></i>	3664	-0.21 (-0.34, -0.07)	-0.05	0.002	0.002
cIMT, <i>mm</i>	3661	-0.002 (-0.003, -0.001)	-0.05	0.002	0.002
CRP, <i>mg/L</i>	3669	-1.01 (-1.02, -1.01)	-0.06	<0.001	0.003
Multivariable linear regression analysis					
Cardiovascular risk factors	n	Unstd. $\beta$ -coeff. (95% CI)	Std. $\beta$ -coeff.	p	R <sup>2</sup>
HDL cholesterol, <i>mmol/L</i> <sup>a</sup>	3609	0.0001 (-0.005, 0.005)	0.00	0.98	0.30
LDL cholesterol, <i>mmol/L</i> <sup>b</sup>	3646	-0.01 (-0.02, -0.002)	-0.04	0.02	0.28
Triglycerides, <i>mmol/L</i> <sup>c</sup>	3609	-0.02 (-0.03, -0.02)	-0.10	<0.001	0.19
Fasting glucose, <i>mmol/L</i> <sup>d</sup>	3605	-0.03 (-0.04, -0.02)	-0.10	<0.001	0.36
HbA1c, % <sup>e</sup>	3599	0.00 (-0.01, 0.01)	-0.002	0.90	0.41
BMI, <i>kg/m<sup>2</sup></i> <sup>f</sup>	3612	-0.20 (-0.25, -0.15)	-0.13	<0.001	0.16
SBP <sup>g</sup>	3609	-0.25 (-0.47, -0.03)	-0.04	0.03	0.03
DBP <sup>h</sup>	3609	-0.14 (-0.25, -0.02)	-0.02	0.02	0.12
eGFR, <i>ml/min x 1.73m<sup>2</sup></i> <sup>i</sup>	3593	-0.37 (-0.51, -0.23)	-0.09	<0.001	0.04
cIMT, <i>mm</i> <sup>j</sup>	3635	0.000 (-0.001, 0.001)	-0.01	0.73	0.07
CRP, <i>mg/L</i> <sup>k</sup>	3603	-1.01 (-1.02, 1.00)	-0.03	0.06	0.07

<sup>a</sup> Gender, smoking, diabetes mellitus, BMI, lipid-lowering drugs, glucose-lowering drugs, hypertension, physical activity, higher education, age (months), alcohol consumption.

<sup>b</sup> Gender, diabetes mellitus, BMI, lipid-lowering drugs, glucose-lowering drugs, higher education, age (months).

<sup>c</sup> Gender, smoking, diabetes mellitus, BMI, lipid-lowering drugs, glucose-lowering drugs, hypertension, physical activity, higher education, age (months), alcohol consumption.

<sup>d</sup> Gender, smoking, BMI, lipid-lowering drugs, glucose-lowering drugs, hypertension, physical activity, higher education, age (months), alcohol consumption.

<sup>e</sup> Gender, smoking, BMI, lipid-lowering drugs, glucose-lowering drugs, hypertension, physical activity, higher education, age (months), alcohol consumption.

<sup>f</sup> Gender, smoking, diabetes mellitus, lipid-lowering drugs, hypertension, physical activity, higher education, age (months), alcohol consumption.

<sup>g</sup> Gender, smoking, diabetes mellitus, BMI, lipid-lowering drugs, physical activity, higher education, age (months), alcohol consumption, antihypertensive drugs.

<sup>h</sup> Gender, smoking, diabetes mellitus, BMI, lipid-lowering drugs, glucose-lowering drugs, physical activity, higher education, age (months), alcohol consumption, antihypertensive drugs.

<sup>i</sup> Gender, smoking, diabetes mellitus, BMI, lipid-lowering drugs, hypertension, physical activity, higher education, age (months), alcohol consumption.

<sup>j</sup> Gender, smoking, BMI, glucose-lowering drugs, hypertension, age (months).

<sup>k</sup> Gender, smoking, diabetes mellitus, BMI, lipid-lowering drugs, hypertension, physical activity, higher education, age (months).

Unstandardized  $\beta$  coefficients (Unstd.  $\beta$ -coeff.) with corresponding 95% confidence intervals (CI), standardized  $\beta$  coefficients (Std.  $\beta$ -coeff.), p-values and explained variance ( $R^2$ ) are given for plasma LA in the univariable analysis and for the fully adjusted multivariable model.

Abbreviations: HDL: High-density lipoprotein. LDL: Low-density lipoprotein. HbA1c: Hemoglobin A1c. BMI: Body mass index. SBP: Systolic blood pressure. DBP: Diastolic blood pressure. eGFR: Estimated glomerular filtration rate. cIMT: Carotid intima-media thickness. CRP: C-reactive protein.

Fig. 1

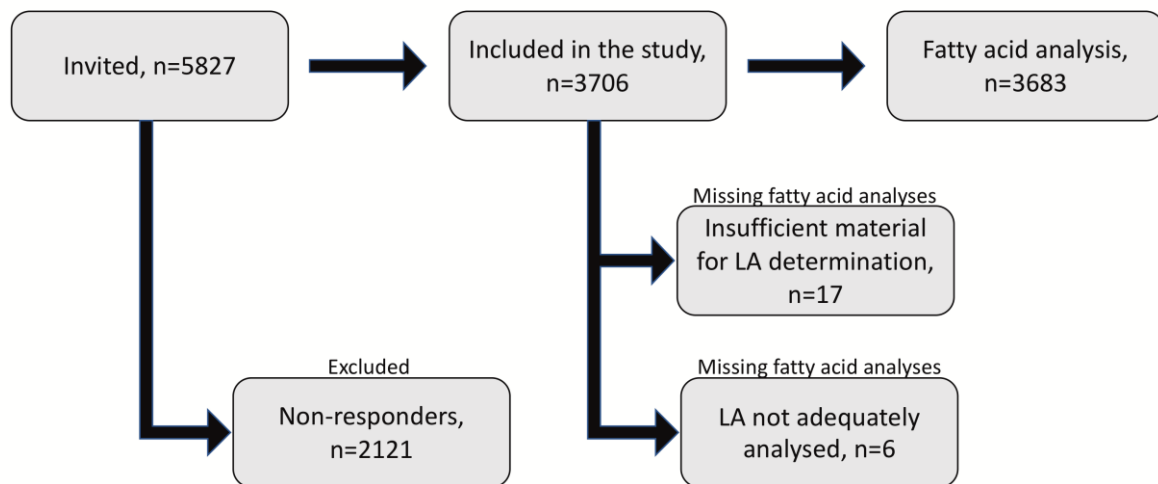


Fig 2.

